Integrated Field, Laboratory, and Modeling Studies to Determine the Effects of Linked Microbial and Physical Spatial Heterogeneity on Engineered Vadose Zone Bioremediation

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Research Objective

In situ bioremediation of contaminants can offer advantages in cost, speed, public acceptance, and final cleanup levels achieved relative to physical removal methods. However, microbial populations in the unsaturated zone are spatially discontinuous and sparse, especially in deep vadose zones and in arid climates with very low moisture and nutrient flux. In addition, there is a lack of knowledge on 1) the ability of microbes to colonize "empty" regions of the vadose zone in response to nutrient delivery and 2) how microbial colonization is controlled by hydrologic and physical features. These issues raise questions about the feasibility of deep vadose zone bioremediation and the accuracy of flow and transport models for vadose zone bioremediation.

The goal of this research is to provide DOE with an increased understanding of the effect of interacting hydrologic and microbiological processes that control the feasibility of engineered bioremediation of chlorinated compounds in heterogeneous, microbially sparse deep vadose zones. The specific objectives are

- to conduct laboratory research on vadose zone microbial colonization processes as a function of hydrologic and physical features, and use the information to develop an improved vadose zone reactive transport model

- to evaluate a gas-phase nutrient delivery approach for enhancing removal of carbon tetrachloride from the vadose zone.

Research Progress and Implications

This report summarizes the progress achieved during 2.5 years of a 3-year project. Research tasks under way at Pacific Northwest National Laboratory are addressing the ability of microbes to colonize uninhabited porous media under static unsaturated conditions. At Oregon State University, researchers are examining the dynamics of microbial metabolic and colonization processes under flowing unsaturated conditions. Both efforts involve understanding how microbial colonization is controlled by porous media water content and particle size.

Static Unsaturated Conditions

Microbial motility—the ability of microorganisms to swim through water or move over surfaces—has been largely discounted as unimportant in microbial colonization of the subsurface. However, no specific studies of microbial motility under vadose zone conditions are reported in the literature. Our results with a carbon-tetrachloride-degrading bacterium and sorted sand of 0.2 to 0.7 mm in diameter show motility-promoted colonization rates of about 2 cm/day under unsaturated conditions in which the calculated average water film thickness is

>20 micrometers. This adds to the existing base of scientific knowledge on phenomena under unsaturated conditions and challenges conventional scientific wisdom. The significance of these results is that nutrient delivery to coarse-grained regions of the unsaturated subsurface could promote colonization by motile bacteria. If the motile bacteria were able to degrade contaminants, colonization of previously "empty" regions would dramatically increase the rate and efficiency of biodegradation.

In the absence of acetate in the columns, there was substantial movement of bacteria via a physical process during the first 10 minutes after bacterial inoculation. This movement was shown to be an artifact (due to pore-scale water redistribution) because bromide tracer, bacteria, and 1-micrometer yellow-green (negatively charged) and bright-blue (neutral charge) microspheres all traveled similar distances in the first 10 minutes. There was little to no additional bacterial movement due to "random motility (i.e., non-chemotactic exploratory motility) in the subsequent 24 hours. In the presence of acetate in the columns, at a given volumetric water content, bacteria generally traveled farther with increasing sand size (0.71-mm-diameter > 0.53 mm > 0.36 mm > 0.21 mm). Bacterial movement was not detected at 5% volumetric water content in the two smallest sands, which had calculated average water film thicknesses of 3 and 6 micrometers. Colonization in the presence of acetate was also not detected in much longer 12-day experiments with 0.10-diameter sand (4-micrometer calculated average water film thickness) at 1.3% volumetric water content. In the presence of acetate in the columns, at a given sand size, bacteria traveled farther with increasing volumetric water content (20% > 15% > 10% > 5%). After 24 hours, bacteria were present at high density throughout the 4-cm-long column at the higher volumetric water contents and larger sand sizes. We are beginning to model bacterial movement in these unsaturated sorted sands using equations that account for the tortuosity of diffusion paths in partially saturated porous media.

Unsaturated Flow Conditions

The ability of microbes to colonize unsaturated porous media in the presence of flow and soluble nutrients was studied in a two-dimensional chamber, 40 cm wide by 60 cm high by 1 cm thick, instrumented to allow periodic visualization of water distribution, nutrient delivery, and microbial activity. Water distribution is visualized by light transmission, nutrient delivery by use of a dye, and microbial activity by periodic addition of salicylate, which causes the genetically engineered bacterium *Pseudomonas fluorescens* HK44 to produce light in the presence of oxygen. A charge-coupled device camera records data at high resolution (1 mm/pixel) and quantifies, in situ, the temporal and spatial interactions between water content, solute transport, and microbial processes. Experiments were conducted with homogenous porous media and with heterogeneous porous media consisting of a wedge of coarse sand within a matrix of finer sand. Homogeneous porous media experiments were funded largely by a previous National Science Foundation project, with EMSP funds used for final analyses and numerical modeling. EMSP funds also served to extend the homogeneous systems to heterogeneous layered systems, which are more typical of Hanford sediments. Our results show that microbial growth causes dynamic changes in flow paths and hydraulic properties in unsaturated systems. They also indicate that physical heterogeneity strongly controls microbial activity and colonization in the unsaturated zone. Results from these experiments are being used as input to parameterization and testing of a two-dimensional finite-difference numerical model for predicting contaminant fate and transport in the vadose zone. This model accounts for water flow, transport of solutes and bacteria, microbial growth and degradation kinetics, gas diffusion, and interphase exchange. The model captures new information on interactions between microbial dynamics and vadose zone processes that can be applied in conjunction with experimental studies to gain insights into, and greater understanding of, these processes and phenomena.

We also have developed a colorimetric readout method for real-time monitoring of gas movement through unsaturated two-dimensional chambers. This work is being extended to track movement of gaseous microbial nutrients in the two-dimensional chambers to investigate relationships between hydraulic processes, gaseous nutrient delivery to microorganisms, and microbiological growth.

Field Studies

Field sampling of the contaminated 216-Z-9 trench on the Hanford Site was conducted in June 2001. Contaminants include carbon tetrachloride and transuranic radionuclides. Twenty-four core samples ranging in depth from 102 to 187 ft below ground surface were collected from two boreholes and analyzed. The field sampling was performed to determine 1) the existence of potential microbial activity throughout the depth profile, 2) the ability of indigenous microorganisms to grow using gaseous sources of nitrogen, phosphorus, and carbon, and 3) the ability of indigenous microorganisms to degrade carbon tetrachloride. Potential activity was detected in 87% of the samples, and facultative anaerobic (denitrifying) bacteria were present in 71% of the samples. Gaseous nutrient injection is a means to stimulate unsaturated zone populations without water addition, thereby reducing the likelihood of transporting contaminants to underlying aquifers. Approximately 75% of the samples removed >10% of one or more gaseous carbon sources, with butane most commonly used (30% of samples), followed by propylene (25%), propane (14%), ethane (8%), and methane (3%). Gaseous nitrogen and phosphorus did not stimulate or inhibit removal of gaseous carbon sources compared to no addition of gaseous nitrogen and phosphorus, indicating these sediments contain adequate levels of nitrogen and phosphorus for substantial microbial growth.

We also have collaborated with the U.S. Geological Survey on its Toxic Substances Hydrology Program in the analysis of samples from the Amargosa Desert Research Site (ADRS) in Beattie, Nevada, to determine if far-field migration of $^{14}C\text{-}CO_2$ may be attributable to microbial activity.

Land disposal of low-level mixed organic-radioactive waste occurred at both the ADRS and the Hanford Site, and the ADRS serves as an analog for understanding processes occurring at Hanford. The data showed that microbial populations and activity were very low away from the burial trenches and the capillary fringe and would not be generating measurable $^{14}C\text{-}CO_2$. The results indicate that microorganisms in and immediately adjacent to the buried waste are generating the $^{14}C\text{-}CO_2$ and that physical transport processes controlled by the site geology are causing far-field migration.

Planned Activities

Microbial Colonization in Static Unsaturated Columns

- Conduct 30-cm-long column experiments at the higher volumetric water contents and larger sand sizes to determine if rates of colonization are maintained over time.

- Conduct experiments with mixtures of sorted sand grain sizes and also with unsorted unsaturated zone sands to determine how much colonization rates are attenuated in porous media that are more geologically common and realistic.

Unsaturated Flow Chambers

- Continue investigation of the spatial and temporal dynamics of microbial processes using gaseous sources of carbon, nitrogen, and phosphorus.

Hanford 216-Z-9 Trench Samples

- Carbon tetrachloride will be spiked into the samples that removed gaseous carbon sources to determine the potential of the hydrocarbon-degraders to remove carbon tetrachloride under unsaturated conditions.

Information Access

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